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Annex to EPO Form 2004, Communication pursuant to Rule 71(3) EPC

Bibliographical data of European patent application No. 04 768 663.9

For the intended grant of the European patent, the bibliographical data are set out below, for information:

Title of invention:
- Zusammensetzung zur Behandlung von Atherosklerose, die Candesartan und Rosuvastatin enthält
- A combination comprising candesartan and rosuvastatin for the treatment of atherosclerosis
- Combinaison comprenant du candesartan et de la rosuvastatine pour le traitement de l'athérosclérose

Classification: INV. A61K31/505 A61K31/4184

Date of filing: 22.09.2004

Priority claimed: GB / 26.09.2003 / GBA0322552

Contracting States* for which fees have been paid: AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

Extension States* for which fees have been paid: AL HR LT LV MK

Applicant(s):**

AstraZeneca UK Limited
15 Stanhope Gate
London,
Greater London W1K 1LN
GB

Inventor(s):

MEHTA, Jay, L.,
Univ. of Arkansas for MedicalScs.
4301 W Markham, Slot 532
Little Rock, AR72205
US

*) If the time limit for the paymant of designation fees according to Rule 39(1) EPC has not yet expired and the applicant has not withdrawn any designation, **all Contracting States/Extension States** are currently still deemed to be designated. See also Rule 71(8) EPC and, if applicable, the above Note to users of the automatic debiting procedure.

**) If two or more applicants have designated different Contracting States, this is indicated here.

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Williams, Anne Rachel Burton
AstraZeneca AB
Global Intellectual Property
151 85 Södertälje
SUÈDE

Application No.	Ref.	Date
04 768 663.9 - 2123	101223-1X EP	09.05.2008
Applicant: AstraZeneca UK Limited		

Communication under Rule 71(3) EPC

You are informed that the Examining Division intends to grant a European patent on the basis of the above application with the text and drawings as indicated below:

In the text for the Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

Description, Pages

1-11 as originally filed

Claims, Numbers

1-8 filed with telefax on 06.03.2008

Drawings, Sheets

1/1 as originally filed

With the following amendments to the above-mentioned documents by the examining division

Description, Pages 1*, 2*, 6**, 7*

Comments

* Second medical use according to EPC 2000
** Method of treatment

A copy of the relevant documents is enclosed

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated Contracting States, the registered name of the applicant and the bibliographic data are shown on the attached EPO Form 2056.

You are requested within a non-extendable period of **four months** of notification of this communication

1. to file 1 set of translations of the claim(s) in the two other EPO official languages;

		EUR
2a.	to pay the fee for grant including the fee for printing up to and including 35 pages; Reference 007	790.00
2b.	to pay the printing fee for the 36th and each subsequent page; number of pages: 0 Reference 008	0.00
3.	to pay the additional claim fee(s) (R. 71(6) EPC); number of claims fees payable: Reference 016	0.00
	Total amount	790.00

The mention of the grant of the patent shall be published in the European Patent Bulletin as soon as possible after the requirements concerning the translation of the claims and the payment of the fees for grant and printing, claims fees, designation fees and renewal fees as laid down in Rule 71(3), (4), (6) and (8) and (9) EPC are fulfilled.

Any divisional applications relating to this European patent application must be filed directly at the European Patent Office in Munich, The Hague or Berlin in accordance with Article 76(1) and Rule 36 EPC **before** the date on which the European Patent Bulletin mentions the grant of the patent (see Art. 97(3) EPC and OJ EPO 2/2002, 112).

If you do not approve the text intended for grant but wish to request amendments or corrections, the procedure described in Rule 71(4) EPC is to be followed.

If this communication is based upon an auxiliary request, and you reply within the time limit set that you maintain the main or a higher ranking request which is not allowable, the application will be refused (Art. 97(2) EPC).

If the enclosed claims contain amendments proposed by the Examining Division, and you reply within the time limit set that you cannot accept these amendments, refusal of the application under Article 97(2) EPC will result if agreement cannot be reached on the text for grant.

In all cases except those of the previous two paragraphs, if the fees for grant and printing or claims fees are not paid, or the translations are not filed, in due time, the European patent application will be deemed to be withdrawn (R. 71(7) EPC).

For all payments you are requested to use EPO Form 1010 or EPO Form 1010E or to refer to the relevant reference number.

After publication, the European patent specification can be downloaded free of charge from the EPO publication server https://publications.european-patent-office.org (OJ EPO 2005, 126).

Upon request in writing each proprietor will receive the certificate for the European patent **together with one copy** of the patent specification provided that the request is filed within the time limit of Rule 71(3) EPC. If such request has been previously filed, it has to be confirmed within the time limit of Rule 71(3) EPC. The requested copy is free of charge. If the request is filed after expiry of the Rule 71(3) EPC time limit, the certificate will be delivered without a copy of the patent specification.

Note on payment of renewal fees

If a renewal fee falls due between notification of the present communication and the proposed date of publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (R. 71(9) EPC).

Under Article 86(2) EPC, the obligation to pay renewal fees to the European Patent Office terminates with the payment of the renewal fee due in respect of the year in which the mention of the grant of the European patent is published.

Filing of translations in the Contracting States

Pursuant to Article 65(1) EPC the following Contracting States require a translation of the specification of the European patent in their/one of their official language(s) (R. 71(10) EPC), **if** this specification is not published in their/one of their official language(s)

- within **three months** of the publication of the mention of the grant:

AT	AUSTRIA	GB	UNITED KINGDOM
BE	BELGIUM	GR	GREECE
BG	BULGARIA	HU	HUNGARY
CH	SWITZERLAND / LIECHTENSTEIN	IT	ITALY
CY	CYPRUS	NL	NETHERLANDS
CZ	CZECH REPUBLIC	PL	POLAND
DE	GERMANY	PT	PORTUGAL
DK	DENMARK	RO	ROMANIA
EE	ESTONIA	SE	SWEDEN
ES	SPAIN	SI	SLOVENIA *
FI	FINLAND	SK	SLOVAKIA
FR	FRANCE	TR	TURKEY

* requires only a translation of the claims

- within **six months** of publication of the mention of the grant:

IE IRELAND

The date on which the mention of the grant of the European patent will be published in the European Patent Bulletin will be indicated in the decision to grant the European patent (EPO Form 2006A).

In case of a valid extension:

The following Extension States require a translation of the claims in their official language within **three months** after publication of the mention of the grant of the European patent:

AL	ALBANIA
HR	CROATIA (requires a translation of the specification)
LT	LITHUANIA
LV	LATVIA
MK	THE FORMER YUGOSLAV REPUBLIC OF MACEDONIA

The translation must be filed with the national Patent Offices of the Contracting or Extension States in accordance with the provisions applying thereto in the State concerned. Further details (e.g. appointment of a national representative or indication of an address for service within the country) are given in the EPO information brochure "National law relating to the EPC" and in the supplementary information updates published in the Official Journal of the EPO, or are available on the EPO website.

Failure to supply such translation to the Contracting or Extension States in time and in accordance with the aforementioned requirements may result in the patent being deemed to be void ab initio in the State concerned.

Important note to users of the automatic debiting procedure

The fees for grant and printing and also any additional claims fees due under Rule 71(6) EPC will be debited automatically on the date of filing of the translation of the (relevant) claims, or on the last day of the period of this communication. However, if the designation fees become due as set out in Rule 71(8) EPC and/or a renewal fee becomes due as set out in Rule 71(9) EPC, these should be paid separately by another permitted means of payment in order not to delay the publication of the mention of grant. The same applies in these circumstances to the payment of extension fees. For further details see the Arrangements for the automatic debiting procedure (AAD) and accompanying Information from the EPO concerning the automatic debiting procedure (Annexes A.1 and A.2 to the Arrangements for deposit accounts (ADA) in Supplement to OJ EPO 10/2007).

Examining Division:

Chairman: Steendijk, Martin
2nd Examiner: Albrecht, Silke
1st Examiner: Damiani, Federica

Lombart, Isabelle
For the Examining Division
Tel. No.: +49 89 2399 - 8779

Enclosure(s): Form 2056
13 Copies of the relevant documents

+++ ATTENTION +++

New amounts of procedural fees as from 01.04.2008 (see OJ EPO 1/2008)!

If additional claims fees (R. 71(6) EPC)* are to be paid and payment is received on or after 01.04.2008, claims fees are only payable from the sixteenth claim onwards. New amount to be paid: EUR 200,- per additional claim.
* to be amended

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80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Application No.: 04 768 663.9

IV.2. Patent classification

The classification indicated on the published patent application remains unchanged. It is as follows:

INV. A61K31/505 A61K31/4184

IV.3. Title of the invention

The title has been changed. It now is as follows:

Zusammensetzung zur Behandlung von Atherosklerose, die Candesartan und Rosuvastatin enthält

A combination comprising candesartan and rosuvastatin for the treatment of atherosclerosis

Combinaison comprenant du candesartan et de la rosuvastatine pour le traitement de l'athérosclérose

IV.4. Documentation

CDOC The following documents not mentioned in the Search Report were cited during the Examination Procedure:

WO 00/45818 A (ASTRAZENECA UK LIMITED) 10 August 2000 (2000-08-10)

WO 01/76573 A (NOVARTIS-ERFINDUNGEN) 18 October 2001 (2001-10-18)

WO 02/058731 A (SCHERING CORP [US]) 1 August 2002 (2002-08-01)

Application No.: 04 768 663.9

Steendijk, Martin
Chairman

Damiani, Federica
1st examiner

Albrecht, Silke
2nd examiner

AstraZeneca

CONFIRMATION COPY

EPO - Munich 21
07. März 2008

6 March 2008

The European Patent Office
D-80298 MÜNCHEN
Germany

Your Ref:
Our Ref: 101223-1X EP/CV

Via Fax: 00 49 89 2399 4465
Confirmation via mail

Re: European Patent Application No. 04768663.9

Dear Sirs,

With reference to the Communication under Art 94(3) EPC dated 13.12.2007.

We enclose an amended claim set in which the following changes have been made:

- Claim 10 has been deleted as requested
- Claims 8 and 9 have been deleted as requested
- The wording "for use in the" has been inserted into claim 1 as requested
- Claims 6 and 7 have been deleted to avoid overlap with other claims
- The remaining claims have been re-ordered and a new claim 5, which is effectively old claim 7 re-worded as for claim 1 has been inserted.
- Clarification of the term "cardiovascular events" has been inserted, using the text on page 1 lines 11-12 as basis
- Original claims 5 and 6 have been re-worded into correct Swiss-type claim format

We have re-ordered the claims as described above for clarity reasons only and we do not believe we have added any subject matter.

We are pleased to note that the Examiner has found the claims to be novel and inventive.

We believe we have addressed all of the points raised by the Examiner and look forward to a Communication under R71(3) EPC. In case the Examiner should be minded to refuse the application, we hereby request Oral Proceedings.

Yours faithfully

Dr Anne Williams
European Patent Attorney
General Authorisation No 41518

Direct Telephone +44 1625 230563

Enc.

AstraZeneca
Global Intellectual Property
SE-151 85 Södertälje
Sweden

Visitors Address
Västra Mälarehamnen 9
Södertälje

Tel +46 8 553 260 00
Fax +46 8 553 288 20
www.astrazeneca.com

Reg Office AstraZeneca AB (publ)
SE-151 85 Södertälje Sweden
Reg No 556011-7821
VAT No SE556011782201

07/03/2008

AZ38

Claims

1. A combination comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof for use in the prevention or treatment of atherosclerosis.

2. A pharmaceutical composition which comprises a combination as claimed in Claim 1 in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

3. Use of a combination as claimed in Claim 1 in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

4. A combination as claimed in Claim 1 wherein candesartan is in the form of candesartan cilexetil.

5. A combination as defined in Claim 1 for use in the prevention of cardiovascular events, such as myocardial infarction, worsening of angina, cardiac arrest, stroke, congestive heart failure and cardiovascular death.

6. A pharmaceutical composition which comprises a combination as claimed in Claim 5, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention of cardiovascular events.

7. Use of a combination as claimed in Claim 5 in the manufacture of a medicament for the prevention of cardiovascular events.

8. A combination as claimed in Claim 5 wherein candesartan is in the form of candesartan cilexetil.

Claims

1. A combination comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof for use in the prevention or treatment of atherosclerosis.

2. A pharmaceutical composition which comprises a combination as claimed in Claim 1 in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

3. Use of a combination as claimed in Claim 1 in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

4. A combination as claimed in Claim 1 wherein candesartan is in the form of candesartan cilexetil.

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6. A pharmaceutical composition which comprises a combination as claimed in Claim 5, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention of cardiovascular events.

7. Use of a combination as claimed in Claim 5 in the manufacture of a medicament for the prevention of cardiovascular events.

8. A combination as claimed in Claim 5 wherein candesartan is in the form of candesartan cilexetil.

AstraZeneca

Fax

To
The European Patent Office

From
Dr Anne Williams

Copies

Subject
European Patent Application No. 04768663.9

Fax number
00 49 89 2399 4465

Fax number
+46 8 553 288 20

Date
6 March 2008

Total pages
3

Reference
Our Ref 101223-1X EP/CV
Your Ref

Split Fax

AstraZeneca
Global Intellectual Property
SE-151 85 Södertälje
Sweden

Tel: +46 8 553 260 00
Fax: +46 8 553 288 20
www.astrazeneca.com

Reg Office AstraZeneca AB (publ)
SE-151 85 Södertälje, Sweden
Reg No 556011-7482
VAT No SE556011748201

AstraZeneca

6 March 2008

The European Patent Office
D-80298 MÜNCHEN
Germany

Your Ref:
Our Ref: 101223-1X EP/CV

Via Fax: 00 49 89 2399 4465
Confirmation via mail

Re: European Patent Application No. 04768663.9

Dear Sirs,

With reference to the Communication under Art 94(3) EPC dated 13.12.2007.

We enclose an amended claim set in which the following changes have been made:

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- Clarification of the term "cardiovascular events" has been inserted, using the text on page 1 lines 11-12 as basis
- Original claims 5 and 6 have been re-worded into correct Swiss-type claim format

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Yours faithfully

Dr Anne Williams
European Patent Attorney
General Authorisation No 41518

Direct Telephone +44 1625 230563

Enc.

AstraZeneca
Global Intellectual Property
SE-151 85 Södertälje
Sweden

Visitors Address
Västra Mälarehamnen 9
Södertälje

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Fax +46 8 553 286 20
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European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Williams, Anne Rachel Burton
AstraZeneca AB
Global Intellectual Property
151 85 Södertälje
SUÈDE

Formalities Officer
Name: Senkel, Heinz
Tel: +49 89 2399 - 6027
or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Damiani, Federica
Tel: +49 89 2399 - 2664

Application No.	Ref.	Date
04 768 663.9 - 2123	101223-1X EP	13.12.2007
Applicant: AstraZeneca UK Limited		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC.

One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

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Damiani, Federica
Primary Examiner
for the Examining Division

Enclosure(s): 4 page/s reasons (Form 2906)
D6:WO0045818; D7:WO0176573; D8:WO02058731

The examination is being carried out on the **following application documents**:

Description, Pages

1-11 as originally filed

Claims, Numbers

1-11 as originally filed

Drawings, Sheets

1/1 as originally filed

1. The present application discloses the combination of candesartan and rosuvastatin and their use for prevention or treatment of atherosclerosis/cardiovascular “events”.

2. Reference is made to the following documents:
Unless otherwise indicated, reference is made to the passages indicated in the search report.
D1: CHEN JIAWEI ET AL: "Marked upregulation of lipoxygenase-1, a receptor for ox-low-density lipoprotein in atherosclerosis, and its total ablation by candesartan and rosuvastatin given concurrently." JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 43, no. 5 Supplement A, 3 March 2004 (2004-03-03), page 498A, XP002319611 & 53RD ANNUAL SCIENTIFIC SESSION OF THE AMERICAN COLLEGE OF CARDIOLOGY; NEW ORLEANS, LA, USA; MARCH 07-10, 2004 ISSN: 0735-1097
D2: WO 2004/096810 A (PFIZER LIMITED; BELL, ANDREW, SIMON; BROWN, DAVID, GRAHAM; FOX, DAVID,) 11 November 2004 (2004-11-11)
D3: EP-A-1 314 425 (SANKYO COMPANY, LIMITED) 28 May 2003 (2003-05-28)
D4: WO 95/26188 A (MERCK & CO., INC; NELSON, EDWARD, B; SWEET,

CHARLES, S) 5 October 1995 (1995-10-05)

D5: PATENT ABSTRACTS OF JAPAN vol. 2002, no. 09, 4 September 2002 (2002-09-04) & JP 2002 145770 A (SANKYO CO LTD), 22 May 2002 (2002-05-22)

D6: WO0045818

D7: WO0176573

D8: WO02058731

Documents D6-D8 were cited by the examining division.

D1 and D2 were published after the claimed priority, the present priority has been checked and has been found valid for the whole subject-matter claimed. Thus, the content of D1 does not form part of the state of the art.
D2 discloses a pharmaceutical composition comprising a second pharmaceutically active agent to be selected from a list comprising also candesartan and rosuvastatin for the treatment of e.g. pulmonary hypertension, hypertension associated with atherosclerosis and diabetes. D2 does not disclose the claimed subject-matter of present application, therefore, it is not relevant for the present opinion.

3. NOVELTY

D3 (EP1314425) relates to pharmaceutical compositions for the prevention or treatment of cardiac failure, the prevention of ischemic coronary heart disease or the prevention of the recurrence of ischemic coronary heart disease, said pharmaceutical compositions containing a HMG-CoA reductase inhibitor selected from the group consisting of pravastatin, simvastatin, lovastatin, pitavastatin and ZD-4522 (rosuvastatin) and an angiotensin II receptor antagonist, e.g. candesartan and optionally further containing a calcium channel blocker (1/[0001]). The combinations of present application (candesartan-rosuvastatin) would arise from selections from two lists.
D4 (W095/26188) discloses the combination of HMG-CoA reductase inhibitor and an angiotensin II receptor antagonist for the treatment of atherosclerosis (1/6-16; claim 1).
D5 (JP20021 45770) is directed to the use of a HMG-CoA reductase inhibitor together with an angiotensin II receptor antagonist for the treatment of heart diseases (abstract).
D7 (WO0176573) discloses combinations of at least two therapeutic components one

selected from the group of AT1-receptor antagonists comprising i.a. candesartan, the other selected from the group of HMG-Co-A reductase inhibitors comprising i.a. rosuvastatin for the treatment of atherosclerosis and coronary heart diseases (claims 1, 2, 4, 9). The combinations of present application (candesartan-rosuvastatin) would arise from selections from two lists.
D8 (WO02058731) discloses combinations comprising at least a compound selected among the group of cardiovascular agents comprising i.a. candesartan and candesartan cilexetil and further comprising a compound selected from the group of the HMG-CoA reductase inhibitors comprising i.a. rosuvastatin for the treatment of vascular conditions (claims 11, 25, 35-37, 47). The combinations of present application (candesartan-rosuvastatin) would arise from selections from two lists.
Accordingly, D3-D5, D7-D8 do not anticipated the subject-matter of present application.

D6 (WO0045818) discloses a combination comprising candesartan and rosuvastatin calcium for the treatment of diabetic neuropathy and diabetes (page 4 line 18).
Accordingly, the combinations of present application for use as medicament are not new.
Therefore, claims 1-11 are considered new if directed to a second medical use of these combinations, accordingly, in the claims the applicant is required to replace the wording "...for the treatment of.." with the wording "...for use in the treatment of.." in conformity with Article 54(5) EPC.
In addition, claim 10 appears to differ from previous claims as it refers to a kit further comprising instructions for use. The applicant is reminded that the content of instructions included in a kit describing the use of the formulations are not considered as technical features, whereas the claims should define the subject-matter by technical features (Rule 43(1) EPC). Accordingly, the applicant is required to abandon current claim 10.

4. INVENTIVE STEP

In as far as the claimed subject matter is new the following observations as to the requirement of inventive step apply:
D3 and D7 which are the closest prior art differ from the present invention only in that they do not suggest the claimed combination as such. They gives a list of HMG-CoA reductase inhibitors and a list of angiotensin II receptor antagonists.
The problem to be solved can be described as how to provide further medicaments

for the treatment of atherosclerosis.
None of the prior art documents explicitly suggests the claimed combinations. Moreover, the applicant has shown an synergistic effect of the combination of candesartan and rosuvastatin (see Fig. and pages 10, 11). Therefore, the use of the claimed combinations for the treatment of atherosclerosis seems to be inventive (Article 56 EPC).

5. FURTHER OBSERVATIONS

5.1 Claims 8,9 refer to a method of treatment of the human/animal body, which is an exception to patentability (Article 53(c) EPC).

5.2 The term “cardiovascular events” is unclear (Article 84 EPC).

P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
(070) 3 40 20 40
FAX (070) 3 40 30 16

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Generaldirektion 1 | Directorate General 1 | Direction générale 1

Williams, Anne Rachel Burton
AstraZeneca AB
Global Intellectual Property
151 85 Södertälje
SUEDE

EPO Customer Services
Tel.: +31 (0)70 340 45 00

Date
31.05.06

Reference	Application No./Patent No.
101223-1X EP	04768663.9 - 2107 PCT/GB2004004120

Applicant/Proprietor
AstraZeneca UK Limited

Notification of European publication number and information on the application of Article 67(3) EPC

The provisional protection under Article 67(1) and (2) EPC in the individual contracting states becomes effective only when the conditions referred to in Article 67(3) EPC have been fulfilled (for further details, see information brochure of the European Patent Office "National Law relating to the EPC" and additional information in the Official Journal of the European Patent Office).

A request has been made for extension of the patent to: AL HR LT LV MK
See Official Journal 1-2/1994 for further information on provisional protection.

Pursuant to Article 158(1) EPC the publication under Article 21 PCT of an international application for which the European Patent Office is a designated Office takes the place of the publication of a European patent application.

The bibliographic data of the above-mentioned Euro-PCT application will be published on 28.06.06 in Section I.1 of the European Patent Bulletin. The European publication number is 1673091.

In all future communications to the European Patent Office, please quote the application number plus Directorate number.

Receiving Section

P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
(070) 3 40 20 40
FAX (070) 3 40 30 16

Europäisches Patentamt | European Patent Office | Office européen des brevets

Generaldirektion 1 | Directorate General 1 | Direction générale 1

Williams, Anne Rachel Burton
AstraZeneca AB
Global Intellectual Property
151 85 Södertälje
SUEDE

EPO Customer Services

Tel.: +31 (0)70 340 45 00

Date
17-05-2006

Reference	Application No./Patent No.
101223-1X EP	04768663.9 - 2107 PCT/GB2004004120

Applicant/Proprietor
AstraZeneca UK Limited

Communication pursuant to Rules 109 and 110 EPC

(1) Amendment of application documents, especially the claims (R. 109 EPC)

The above mentioned international (Euro-PCT) application has entered the European phase, or can do so, once the necessary conditions are fulfilled.

Under Articles 28, 41 PCT, Rules 52, 78 PCT and Rule 86(2) to (4) EPC, the applicant may amend the application documents after receiving the international search report.

Whether or not he has already done so, he now has a further opportunity to file amended claims or other application documents within a non-extendable time limit of one month after notification of the present communication (R. 109 EPC).

The claims applicable on expiry of the above time limit, i.e. those filed on entry into the European phase or in response to the present communication, will form the basis for the calculation of any claims fee to be paid (see page 2) and for any supplementary search to be carried out under Article 157(2) EPC (R. 109 EPC).

--2/

Registered letter
EPO Form 1226 01.02 EUCL

(2) Claims fees under Rule 110 EPC

If the application documents on which the European grant procedure is to be based comprise more than ten claims, a claims fee shall be payable for the eleventh and each subsequent claim within the period provided for in Rule 107(1) EPC.

☐ Based on the application documents currently on file, all necessary claims fees have already been paid (or the documents do not comprise more than 10 claims).

☑ All necessary fees will be/have been debited automatically according to the automatic debit order.

☐ The claims fees due for the claims ..... to ..... were not paid within the above-mentioned period.

Any non-paid claims fee, either based on the current set of claims or on any amended claims to be filed pursuant to Rule 109 EPC (see page 1), may still be validly paid within a non-extendable period of grace of **one month** after notification of this communication.

If a payment is made for only some of the claims, it must be indicated for which claims it is intended. If a claims fee is not paid in due time, the claim concerned is deemed to be abandoned (R. 110(4) EPC).

If claims fees have already been paid, but on expiry of the above-mentioned time limit there is a new set of claims containing fewer fee-incurring claims than previously, the claims fees in excess of those due under Rule 110(2), 2nd sentence, EPC will be refunded (R. 110(3) EPC).

You are reminded that any supplementary search under Article 157(2) EPC will relate only to the last set of claims applicable on expiry of the above time limit AND will be confined to those fee-incurring claims for which fees have been paid in due time.

The fee for the eleventh and each subsequent claim is EUR 45,00.

Receiving Section

Europäisches Patentamt • European Patent Office • Office européen des brevets

To the European Patent Office

Entry into the European phase (EPO as designated or elected Office)

European application number	EP04768663.9
PCT application number	PCT/GB2004/004120
PCT publication number	WO2005030215
Applicant's or representative's reference	101223-1X EP
1. Applicant	
Particulars of the applicant(s) are contained in the international publication or were recorded by the International Bureau subsequent to the international publication.	☑
Changes which have not yet been recorded by the International Bureau are set out here:	☐
Address for correspondence	
2. Representative 1	
This is the representative who will be listed in the Register of European Patents and to whom notifications will be made	
Name	Global Intellectual Property AstraZeneca AB
Registration No	4695960.7
Address of place of business	
	Södertälje, SE-151 85
	Sweden
Telephone	+46 8 553 260 00
Fax	+46 8 553 288 20
e-mail	patents@astrazeneca.com
Any additional representative(s) is/are listed here:	☐
3. General Authorisation:	
An individual authorisation is attached.	☐
A general authorisation has been registered under No:	☑ 41518
A general authorisation has been filed, but not yet registered.	☐
The authorisation filed with the EPO as PCT receiving Office expressly includes the European phase.	☐
4. Request for examination	
Examination of the application under Art. 94 EPC is hereby requested. The examination fee is being (has been, will be) paid.	☑
Request for examination in an admissible non-EPO language:	☐
5. Copies	
One or more additional sets of copies of the documents cited in the supplementary European search report are hereby requested.	☐
Number of additional sets of copies	
6. Documents intended for proceedings before the EPO	
6.1 Proceedings before the EPO as designated Office (PCT I) are to be based on the following documents:	
the application documents published by the International Bureau (with all claims, description and drawings), where applicable with amended claims under Art. 19 PCT	☑

unless replaced by the amendments attached.	☐
Where necessary, clarifications should be attached as `Other Documents`	
6.2 Proceedings before the EPO as elected Office (PCT II) are to be based on the following documents:	
the documents on which the international preliminary examination report is based, including any annexes	☐
unless replaced by the amendments attached.	☐
Where necessary, clarifications should be attached as `Other Documents`	
If the EPO as International Preliminary Examining Authority has been supplied with test reports, these may be used as the basis of proceedings before the EPO.	☐
7. Translations	
Translations in one of the official languages of the EPO (English, French, German) are attached as crossed below:	☐
* In proceedings before the EPO as designated or elected Office (PCT I + II):	
Translation of the international application (description, claims, any text in the drawings) as originally filed, of the abstract as published and of any indication under Rule 13bis.3 and 13bis.4 PCT regarding biological material	☐
Translation of the priority application(s)	☐
It is hereby declared that the international application as originally filed is a complete translation of the previous application (Rule 38(5) EPC)	☐
* In addition, in proceedings before the EPO as designated Office (PCT I):	
Translation of amended claims and any statement under Art. 19 PCT, if the claims as amended are to form the basis for the proceedings before the EPO (see Section 6).	☐
* In addition, in proceedings before the EPO as elected office (PCT II):	
Translation of annexes to the international preliminary examination report	☐
8. Biological material	
The invention relates to and/or uses biological material deposited under Rule 28 EPC.	☐
The particulars referred to in Rule 28(1)(c) EPC (if not yet known, the depository institution and the identification reference(s)) [number, symbols, etc.] of the depositor) are given in the international publication or in the translation submitted under Section 7 on:	☐
page(s) / line(s)	
A copy of the receipt(s) of deposit issued by the depositary institution	
is attached	☐
will be filed at a later date	☐
A waiver of the right to an undertaking from the requester pursuant to Rule 28(3) EPC is attached.	☐
9. Nucleotide and amino acid sequences	
The items required under Rules 5.2 and 13ter PCT and Rule 111(3) EPC have already been furnished to the EPO.	☐
The sequence listing as part of the description is attached in PDF format.	☐
The sequence listing does not include matter that goes beyond the content of the application as filed.	☐
In addition, the sequence listing data is attached in computer-readable form in accordance with WIPO Standard 25.	☐
The sequence listing data in computer-readable form in accordance with WIPO Standard 25 is identical to the sequence listing in PDF format.	☐
10. Designation fees	
10.1 It is currently intended to pay seven times the amount of the designation fee. The designation fees for all the EPC contracting states designated in the international application are thereby deemed to have been paid (Art. 2 No. 3 RFees).	☑
AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PL	

PT RO SE SI SK TR

10.2 It is currently intended to pay fewer than seven designation fees for the following EPC contracting states designated in the international application: ☐

10.3 It is requested that no communication under Rules 85a(1) or 69(1) need be notified in respect of the contracting states not indicated. If an automatic debit order has been issued, the EPO is authorised, on expiry of the basic period under Article 79(2), to debit seven times the amount of the designation fee. If less than seven states are indicated, the EPO shall debit designation f ees only for those states, unless it is instructed to do otherwise before expiry of the basic period. ☑

11. Extension of the European patent

This application is also considered as being a request for extension to all the non-contracting states to the EPC designated in the international application with which "extension agreements" were in force on the date of filing the international application. However, the extension only takes effect if the prescribed extension fee is paid. ☑

It is currently intended to pay the extension fee for the following states:

AL HR MK LT LV

12. List of enclosed documents

	Description of document	Original file name	Assigned file name

13. Automatic debit order ☑

Currency: EUR

The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account any fees and costs falling due.

Deposit account number: 28100023

Account holder: AstraZeneca AB

14. Reimbursements (if any) should be made to the following EPO deposit account: ☑

Number and account holder: AstraZeneca AB, 28100023

15. Fees

		Factor applied	Fee schedule	Amount to be paid
15-1	005 Designation fee	7	75.00	525.00
15-2	006 Examination fee	1	1 430.00	1 430.00
15-3	015 Claims fee	1	40.00	40.00
15-4	020 Basic national fee for an international application	1	90.00	90.00
15-5	033 Renewal fee for the 3rd year	1	380.00	380.00
15-6	402 Extension fee for Lithuania (Memberstate as from 1.12.2004)	1	102.00	102.00
15-7	403 Extension fee for Latvia	1	102.00	102.00
15-8	404 Extension fee for Albania	1	102.00	102.00
15-9	406 Extension fee for former Yugoslav Republic of Macedonia	1	102.00	102.00
15-10	407 Extension fee for Croatia	1	102.00	102.00
	Total:		EUR	2 975.00

16. Annotations

17. Signature(s) of applicant(s) or representative

Place: Macclesfield, Cheshire, UK

Date: 19.April 2006

Signed by: /Anne Williams/

Capacity: (Employee of AstraZeneca UK Limited)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the form for entry into the European phase (EPO as designated or elected Office) as follows:

Submission number	114583	
PCT application number	PCT/GB2004/004120	
Date of receipt	19 April 2006	
Receiving Office	European Patent Office, The Hague	
Your reference	101223-1X EP	
Applicant		
Country		
Documents submitted	package-data.xml	epf1200.pdf (3 p.)
	ep-euro-pct.xml	application-body.xml
Submitted by	Subject: Macclesfield, Cheshire, UK; Issuer: AstraZeneca UK Limited	
Method of submission	Online	
Date and time receipt generated	19 April 2006, 12:25:25 (CEST)	
Digest	45:00:98:64:9B:EE:35:89:84:8B:0F:B4:8B:18:09:AB:53:79:B6:CD	

/European Patent Office/

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 101223-1 WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/GB2004/004120	International filing date *(day/month/year)* 22 September 2004 (22.09.2004)	Priority date *(day/month/year)* 26 September 2003 (26.09.2003)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant ASTRAZENECA UK LIMITED		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 27 March 2006 (27.03.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35	Authorized officer Dorothée Mülhausen Telephone No. +41 22 338 87 40

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

7/4
(67)

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/GB2004/004120	International filing date *(day/month/year)* 22.09.2004
Priority date *(day/month/year)* 26.09.2003	
International Patent Classification (IPC) or both national classification and IPC A61K31/505, A61K31/4184	
Applicant ASTRAZENECA UK LIMITED	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Heller, D

Telephone No. +49 89 2399-8746

10/04/2006

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/004120

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Form PCT/ISA/237 (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/004120

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 8, 9

because:

☒ the said international application, or the said claims Nos. 8, 9 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Form PCT/ISA/237 (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/004120

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-7, 10, 11
	No:	Claims	8,9 (see section V)

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Form PCT/ISA/237 (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/GB2004/004120

Section III:

Claims 8 and 9 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Section V:

Subject-matter

The present application discloses the combination of candesartan and rosuvastatin and their use (for prevention or treatment of atherosclerosis/cardiovascular "events").

Prior art

Reference is made to the following documents:

D3 (EP1314425) relates to pharmaceutical compositions for the prevention or treatment of cardiac failure, the prevention of ischemic coronary heart disease or the prevention of the recurrence of ischemic coronary heart disease, said pharmaceutical compositions containing a HMG-CoA reductase inhibitor selected from the group consisting of pravastatin, simvastatin, lovastatin, pitavastatin and ZD-4522 and an angiotensin II receptor antagonist and optionally further containing a calcium channel blocker (1/[0001]).

D4 (WO95/26188) discloses the combination of HMG-CoA reductase inhibitor and an angiotensin II receptor antagonist for the treatment of atherosclerosis (1/6-16; claim 1)

D5 (JP2002145770) is directed to the use of a HMG-CoA reductase inhibitor together with an angiotensin II receptor antagonist for the treatment of heart diseases (abstract).

The numbering results from the order of citations found in the Search Report.

Novelty

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/GB2004/004120

In view of the prior art as summarized above the subject-matter of claims 1 to 11 seems to be novel und Article 33 (2) PCT.

Inventive step

The subject-matter of claims 1 to 11 seems to involve an inventive step in the sense of Article 33 (3) PCT.

D3 which is the closest prior art differs from the present invention only in that it does not suggest the claimed combination as such. It gives a list of HMG-CoA reductase inhibitors and angiotensin II receptor antagonists.

The problem to be solved can be described as how to provide further medicaments for the treatment of atherosclerosis.

None of the prior art documents suggests the claimed combination. However, the applicant has shown an synergistic effect of the combination of candesartan and rosuvastatin (= ZD-4522) (see Fig.). Therefore, the claimed combination seems to be inventive.

Clarity

The term "cardiovascular events" is unclear (Article 6 PCT).

Industrial applicability

For the assessment of the present claims 8 and 9 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/GB2004/004120

Section VI:

Although D1 and D2 (Chen et al. and WO2004/96810) do not constitute prior art within the meaning of Rule 64.1 (b) PCT, it appears to disclose all the features of claims 1 to 11 of the present application.

P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
(070) 3 40 20 40
FAX (070) 3 40 30 16

Europäisches Patentamt | European Patent Office | Office européen des brevets

Generaldirektion 1 | Directorate General 1 | Direction générale 1

ASTRAZENECA
Global Intellectual Property
S-151 85 Sodertalje
SUEDE

EPO Customer Services
Tel.: +31 (0)70 340 45 00

Date
10.02.06

Reference	Application No./Patent No.
	04768663.9 - 2123 PCT/GB2004004120

Applicant/Proprietor
AstraZeneca UK Limited

Entry into the European phase before the European Patent Office

These notes describe the procedural steps required for entry into the European phase before the European Patent Office (EPO). You are advised to read them carefully: failure to take the necessary action in time can lead to your application being deemed withdrawn.

1. The above-mentioned international patent application has been given European application No. **04768663.9**.

2. Applicants **without** a residence or their principal place of business in an EPC contracting state may themselves initiate European processing of their international applications, provided they do so before expiry of the 31st month from the priority date (see also point 6 below).

   During the European phase before the EPO as designated or elected Office, however, such applicants must be represented by a professional representative (Arts. 133(2) and 134(1), (7) EPC).

   Procedural acts performed after expiry of the 31st month by a professional representative who acted during the international phase but is not authorised to act before the EPO have no legal effect and therefore lead to loss of rights.

   Please note that a professional representative authorised to act before the EPO and who acted for the applicant during the international phase does not automatically become the representative for the European phase. Applicants are therefore strongly advised to appoint in good time any representative they wish to initiate the European phase for them; otherwise, the EPO has to send all communications direct to the applicant.

3. Applicants **with** a residence or their principal place of business in an EPC contracting state are not obliged to appoint, for the European phase before the EPO as designated or elected Office, a professional representative authorised to act before the EPO.
   However, in view of the complexity of the procedure it is recommended that they do so.

4. Applicants and professional representatives are also strongly advised to initiate the European phase using EPO Form 1200 (available free of charge from the EPO). This however is not compulsory.

5. **To enter the European phase before the EPO**, the following acts must be performed.
(N.B.: Failure validly to do so will entail loss of rights or other adverse legal consequences.)

5.1 If the EPO is acting as **designated** or **elected** Office (Arts. 22(1)(3) and 39(1) PCT respectively), applicants must, within 31 months from the date of filing or (where applicable) the earliest priority date:

a) Supply a translation of the international application into an EPO official language, if the International Bureau did not publish the application in such a language (Art. 22(1) PCT and Rule 107(1)(a) EPC).
If the translation is not filed in time, the international application is deemed withdrawn before the EPO (Rule 108(1) EPC).
This loss of rights is deemed not to have occurred if the translation is then filed within a two-month grace period as from notification of an EPO communication, provided a surcharge is paid at the same time (Rule 108(3) EPC).

b) Pay the national basic fee (EUR 160,00) and, where a supplementary European search report has to be drawn up, the search fee (EUR 960,00 ; Rule 107(1)(c) and (e) EPC).

c) If the time limit under Article 79(2) EPC expires before the 31-month time limit, pay the designation fee (EUR 75,00) for each contracting state designated (Rule 107(1)(d) EPC).

d) If the time limit under Article 94(2) EPC expires before the 31-month time limit, file the written request for examination **and** pay the examination fee (EUR 1430,00 ; Rule 107(1)(f) EPC).

e) Pay the third-year renewal fee (EUR 380,00) if it falls due before expiry of the 31-month time limit (Rule 107(1)(g) EPC).

If the fees under (b) to (d) above are not paid in time, or the written request for examination is not filed in time, the international application is deemed withdrawn before the EPO, or the contracting-state designation(s) in question is (are) deemed withdrawn (Rule 108(1) and (2) EPC). However, the fees may still be validly paid within a two-month grace period as from notification of an EPO communication, provided the necessary surcharges are paid at the same time (Rule 108(3) EPC). For the renewal fee under (e) above, the grace period is **six** months from the fee's due date (Article 86(2) EPC).

5.2 If the application documents on which the European grant procedure is to be based comprise more then ten claims, a claims fee is payable within the 31-month time limit under Rule 107(1) EPC for the eleventh and each subsequent claim (Rule 110(1) EPC). The fee can however still be paid within a one-month grace period as from notification of an EPO communication pointing out the failure to pay (Rule 110(2) EPC).

6. If the applicant had a representative during the application's international phase, the present notes will be sent to the representative, asking him to inform the applicant accordingly.

All subsequent communications will be sent to the applicant, or - if the EPO is informed of his appointment in time - to the applicant's European representative.

7. For more details about time limits and procedural acts before the EPO as designated and elected Office, see the EPO brochure

   How to get a European patent
   Guide for applicants - Part 2
   PCT procedure before the EPO - "Euro-PCT"

   This brochure, the list of professional representatives before the EPO, Form 1200 and details of the latest fees are now all available on the Internet under

   http://www.european-patent-office.org

RECEIVING SECTION

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 April 2005 (07.04.2005)

PCT

(10) International Publication Number
WO 2005/030215 A3

(51) International Patent Classification[7]: A61K 31/505, 31/4184

(21) International Application Number:
PCT/GB2004/004120

(22) International Filing Date:
22 September 2004 (22.09.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0322552.1 26 September 2003 (26.09.2003) GB

(71) Applicant *(for all designated States except US)*: **ASTRAZENECA UK LIMITED** [GB/GB]; 15 Stanhope Gate, London, Greater London W1K 1LN (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **MEHTA, Jay, Lal** [US/US]; University of Arkansas for Medical Sciences, 4301 W Markham, Slot 532, Little Rock, AR 72205 (US).

(74) Agent: **ASTRAZENECA**; Global Intellectual Property, S-151 85 Sodertalje (SE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
2 June 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERAPEUTIC TREATMENT

(57) Abstract: A combination comprising candesartan and rosuvastatin for the prevention or treatment of arteriosclerosis and for the prevention of cardiovascular events is described.

INTERNATIONAL SEARCH REPORT

ISR

Intern bnal Application No EP04768663
PCT/GB2004/004120

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/505 A61K31/4184

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHEN JIAWEI ET AL: "Marked upregulation of lipoxygenase-1, a receptor for ox-low-density lipoprotein in atherosclerosis, and its total ablation by candesartan and rosuvastatin given concurrently." JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 43, no. 5 Supplement A, 3 March 2004 (2004-03-03), page 498A, XP002319611 & 53RD ANNUAL SCIENTIFIC SESSION OF THE AMERICAN COLLEGE OF CARDIOLOGY; NEW ORLEANS, LA, USA; MARCH 07-10, 2004 ISSN: 0735-1097 abstract	1-11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2005	18/03/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heller, D

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2004/096810 A (PFIZER LIMITED; BELL, ANDREW, SIMON; BROWN, DAVID, GRAHAM; FOX, DAVID,) 11 November 2004 (2004-11-11) claims 1-22	1-11
Y	EP 1 314 425 A (SANKYO COMPANY, LIMITED) 28 May 2003 (2003-05-28) page 1, paragraph 1	1-11
Y	WO 95/26188 A (MERCK & CO., INC; NELSON, EDWARD, B; SWEET, CHARLES, S) 5 October 1995 (1995-10-05) page 1, lines 6-16 claim 1	1-11
Y	PATENT ABSTRACTS OF JAPAN vol. 2002, no. 09, 4 September 2002 (2002-09-04) & JP 2002 145770 A (SANKYO CO LTD), 22 May 2002 (2002-05-22) abstract	1-11

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		WO 0217913 A1	07-03-2002
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P01/7700 0.00-0322552.1

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1. Your reference 101223-1 GB

2. Patent application number *(The Patent Office will fill in this part)* 0322552.1 — 26 SEP 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca UK Limited
15 Stanhope Gate
London
W1K 1LN

Patents ADP number *(if you know it)* 78964467002

If the applicant is a corporate body, give the country/state of its incorporation: England

4. Title of the invention

THERAPEUTIC TREATMENT

5. Name of your agent *(if you have one)*

Kevin BILL

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

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Signature [signature] Date 25.09.03

12. Name and daytime telephone number of person to contact in the United Kingdom: Jennifer Bennett - 01625 230148

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THERAPEUTIC TREATMENT

The present invention relates to a combination comprising candesartan and rosuvastatin.

The present invention further relates to pharmaceutical compositions comprising the combination mentioned hereinbefore. The present invention further relates to the use of a combination mentioned hereinbefore in the prevention or treatment of atherosclerosis.

Atherosclerosis is a condition mediated by complex pathological processes which result in irregularly distributed lipid deposits in the arteries and is a major contributory factor to coronary heart disease. A reduction in atherosclerosis is therefore a major target for reducing the number of cardiovascular events for example, myocardial infarction, worsening of angina, cardiac arrest, stroke, congestive heart failure and cardiovascular death.

Dyslipidemia, particularly increased plasma level of low-density lipoprotein (LDL) is one of the major risk factors in atherosclerosis. Clinical studies have demostrated that reducing plasma LDL level with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, commonly known as statins, results in a lower risk of cardiovascular events.

Activation of the renin-angiotensin system (RAS) may be considered another important risk factor in atherosclerosis. Activation of RAS with the formation of angiotensin (II) (A (II)) and the activation of A (II) receptors have been implicated in atherogenesis, plaque rupture, myocardial ischemic dysfunction and congestive heart failure (Singh and Mehta, Arch Intern Med, 2003, vol 163, 1296-1304).

We have surprisingly found that the combination of the A(II) antagonist candesartan and the HMG CoA reductase inhibitor rosuvastatin has a synergistic effect in the reduction of atherosclerosis. This synergistic effect appears to arise from synergistic inhibition of expresssion of a number of inflammatory mediators involved in the RAS (for example CD40 and metalloproteinases) and/or inhibition of the expression of the receptor LOX-1 (which is a receptor for oxidised LDL on endothelial cells). The synergistic effect provides strong evidence for cross-talk between the RAS and dyslipidemia in atherogenesis.

In one aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for the prevention or treatment of atherosclerosis.

In one aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for the prevention of cardiovascular events.

Such a combination may also be useful in the treatment or prevention of other diseases associated with these mediators, for example in inflammatory diseases or conditions, such as ischemia-reperfusion injury (to the heart, brain, kidneys, lungs and liver), radiation-induced injury, burn injury and peripheral vascular disease,

Candesartan may suitably be in the form of candesartan, or in the pro-drug form candesartan cilexetil. These forms may be formulated with a further agent such as a diuretic such as hydrochlorothiazide (for example, as marketed as 'Atacand Plus').

Where herein candesartan is referred to, this includes both candesartan and candesartan cilexetil.

Preferably the calcium salt of rosuvastatin, which may be referred to as rosuvastatin calcium, is used in the various aspects of the present invention.

Herein, where the term "combination" is used it is to be understood that this refers to simultaneous, separate or sequential administration. In one aspect of the invention "combination" refers to simultaneous administration. In another aspect of the invention "combination" refers to separate administration. In a further aspect of the invention "combination" refers to sequential administration. Where the administration is sequential or separate, the delay in administering the second component should be such that both agents are present in the body so as to produce the synergistic effect of the combination.

In a further aspect of the invention is provided a pharmaceutical composition which comprises candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

In a further aspect of the invention is provided a pharmaceutical composition which comprises candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or reduction of risk of cardiovascular events.

The compositions described herein may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream, for

rectal administration for example as a suppository or the route of administration may be by direct injection into the tumour or by regional delivery or by local delivery. In other embodiments of the present invention the compounds of the combination treatment may be delivered endoscopically, intratracheally, intralesionally, percutaneously, intravenously, subcutaneously, intraperitoneally or intratumourally. In general the compositions described herein may be prepared in a conventional manner using conventional excipients or carriers that are well known in the art.

Suitable pharmaceutically-acceptable excipients or carriers for a tablet formulation include, for example, inert excipients such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or alginic acid; binding agents such as gelatin or starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl 4-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid excipient, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Candesartan is commercially available as 'Atacand' and 'Atacand Plus'. Rosuvastatin calcium is commercially available as 'Crestor'. Suitable formulations for the present invention include those which are commercially available.

Suitable dosages of each component of the combination are those of the marketed commercial products. Alternatively, the synergy between the components may allow a lower dosage of one or both components to be used. For example, a dose of 4mg, 8mg, 16mg, 32mg, or up to 160mg of candesartan in combination with a dose of 80mg, 40mg, 20mg, 10mg, 5mg or 2.5mg of rosuvastatin may be used. It will be understood that any one of the doses of candesartan may be combined with any suitable dose of rosuvastatin.

It will be appreciated that the pharmaceutical composition according to the present invention includes a composition comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier. Such a composition, for example in a single

oral formulation conveniently provides the therapeutic combination product of the invention for simultaneous administration in the prevention or treatment of atherosclerosis.

Preferably the two components of the combination are both administered orally.

Preferably the two components of the combination are administered as a single oral formulation.

Preferably the combination is formulated for once-a-day dosing.

The dosages and schedules described hereinbefore may be varied according to the particular disease state and the overall condition of the patient. For example, it may be necessary or desirable to reduce the above-mentioned doses of the components of the combination treatment in order to reduce toxicity. Dosages and schedules may also vary if, in addition to a combination treatment of the present invention, one or more additional chemotherapeutic agents are used. Scheduling can be determined by the practitioner who is treating any particular patient using his professional skill and knowledge.

A pharmaceutical composition according to the present invention also includes separate compositions comprising a first composition comprising candesartan or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier, and a second composition comprising rosuvastatin or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier. Such a composition conveniently provides the therapeutic combination of the invention for sequential or separate administration in the synergistic prevention or treatment of atherosclerosis but the separate compositions may also be administered simultaneously.

In another aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use as a medicament for the prevention or treatment of atherosclerosis.

In another aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use as a medicament for the prevention of cardiovascular events.

In another aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament.

In another aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically

acceptable salt thereof, for use in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

In another aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for the prevention of cardiovascular events.

In a further aspect of the invention is provided a method of preventing or treating atherosclerosis in a warm-blooded animal, such as man, which comprises administering a combination of candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof.

In a further aspect of the invention is provided a method of preventing cardiovascular events in a warm-blooded animal, such as man, which comprises administering a combination of candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According to a further aspect of the present invention there is provided a kit comprising a combination of candesartan or a pharmaceutically acceptable salt thereof, and rosuvastatin; or a pharmaceutically acceptable salt thereof ,optionally with instructions for use in the prevention or treatment of atherosclerosis.

According to a further aspect of the present invention there is provided a kit comprising:

a) candesartan in a first unit dosage form;

b) rosuvastatin; in a second unit dosage form; and

c) container means for containing said first and second dosage forms; and optionally

d) with instructions for use in the prevention or treatment of atherosclerosis.

According another aspect of the present invention there is provided a method of inhibiting expression of CD40 and/or metalloproteinases (MMPs) by administering a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According another aspect of the present invention there is provided a method of treating atherosclerotic patients by inhibition of expression of CD40 and/or metalloproteinases (MMPs) by administering an amount of a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof suitable for inhibition of expression of CD40 and/or metalloproteinases (MMPs).

According another aspect of the present invention there is provided a method of inhibiting expression of LOX-1 by administering a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According another aspect of the present invention there is provided a method of treating atherosclerotic patients by inhibition of expression of LOX-1 by administering an amount of a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof suitable for inhibition of expression of LOX-1.

Materials and Methods

Animal Model

Five pairs of C57BL/6J mice and three pairs of homozygous apo-E knockout mice (on C57BL/6J background) were obtained from Jackson Laboratories (Bar Harbor, ME). They were bred by brother-sister mating and housed in a room lit from 6:00 AM to 6:00 PM and kept at 21°C. The C57BL/6J mice (n=10) were continued on regular diet for the entire study period. The apo-E knockout mice were divided into four groups. Group1 (n=10)animals were given high-cholesterol diet (1% cholesterol) alone for 12 weeks since the age of 6 weeks; Group2 (n=10) animals were given high-cholesterol diet with candesartan (1mg/kg/d) for 12 weeks since the age of 6 weeks; Group3 (n=10) animals were given high-cholesterol diet with the rosuvastatin (1mg/kg/d) for 12 weeks since the age of 6 weeks; Group 4 (n=10) were given high-cholesterol diet with candesartan (1mg/kg/d) and rosuvastatin (1mg/kg/d) for 12 weeks since the age of 6 weeks.

At the end of 12-week-treatment, the mice were sacrificed and subject to studies described below. All experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee of University of Arkansas for Medical Sciences.

Quantitative Analysis of Atherosclerotic Plaques

At the end of 12-week-treatment, 5 mice from each group were euthanized and the aortas were separated from surrounding tissues. After removal of the adventitial fat tissue, the aortas were opened longitudinally from the aorta arch to the iliac bifurcation, and fixed in

10% formalin for 24 hours. Then the aortas were rinsed in 70% alcohol briefly, stained with Sudan IV solution for 15 minutes, differentiated in 80% alcohol for 20 minutes and washed in running water for 1 hour (25). The aortas were mounted and their pictures were taken with a camera connected to a dissection microscope. The images were analyzed by soft ware (Image Pro Plus, Media Cybernetics) as described previously (26).

RNA Preparation and Analysis by RT-PCR

At the end of 12-week-treatment, 5 mice from each group were euthanized and the aortas (from aorta arch to iliac bifurcation) were separated from surrounding tissues and stored on dry ice. Each aorta was cut into four segments, two of which were used to extract total RNA with the single-step acid-guanidinium thiocyanate-phenol-chloroform method as described earlier (27). One microgram of total RNA was reverse transcripted into cDNA with oligo-dT (Promega, Madison, WI, U.S.A.) and Maloney murine leukemia virus (M-MLV) reverse transcription (Promega) at 42°C for 1 hour. Two microliters of reverse transcription (RT) material was amplified with Taq DNA polymerase (Promega) and a primer pair specific to mouse LOX-1, CD40 or MMPs (MMP-1, -2, -9). For mouse LOX-1, forward primer: 5′-TTACTCTCCATGGTGGTGCC-3′, reverse primer: 5′-AGCTTCTTCTGCTTGTTGCC-3′ were used. 30 cycles of polymerase chain reaction (PCR) were performed at 94°C for 40 seconds (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). The size of polymerase chain reaction (PCR) product was 193 base pairs. For mouse CD40, forward primer 5′-GTTTAAAGTCCCGGATGCGA-3′ and reverse primer 5′-CTCAAGGCTATGCTGTCTGT-3′ were used. 35 cycles of polymerase chain reaction (PCR) were performed at 94°C for 1 minute (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). The size of PCR product was 408 base pairs. For mouse MMP-1, forward primer 5′-GGACTCTCCCATTCTTAATGA T-3′ and reverse primer 5′-CCTCTTTCTGGATAACATCATCA AC-3′ were used. For mouse MMP-2, forward primer 5′-ATCAAGGGGATCCAGGAGC-3′ and reverse primer 5′-GCAGCGATGAAG ATGATAG-3′ were used. For mouse MMP-9, forward primer 5′-AGTTTGGTGTCGCGGAGCAC-3′ and reverse primer 5′-TACATGAGCGCTTCCGGCAC-3′ were used. For all MMPs, 35 cycles of PCR were performed at 94°C for 1 minute (denaturation), 58°C for 1 minute (annealing), and 75°C for 1 minute (extension). The sizes of PCR product were 627, 718 and 753 base pairs, respectively. A primer pair specific to mouse β-actin was used as housekeeping gene (forward primer: 5′-TTCTACAATGAGCTGCGTTG-3′, reverse primer: 5′-CACTGTGTTGGCATAGAGGTC-

3'). 30 cycles were used at 94°C for 30 seconds (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). PCR product was 560 base pairs. The reverse transcription PCR (RT-PCR)-amplified sample was visualized on 1.5% agarose gel using ethidium bromide.

Protein Preparation and Analysis by Western Blot

Each mouse aorta was cut into four segments. Two of them were used to extract RNA, and the remaining two were used to extract protein as described previously (14). In brief, the aortic tissues were homogenized and lysed in lysis buffer, then centrifuged at 4000 rpm for 10 minutes at 4°C. The lysate proteins from aortas (20 µg/lane) were separated by 10% SDS-PAGE, and transferred to nitrocellulose membranes. After incubation in blocking solution (5% non-fat milk, Sigma), membranes were incubated with 1:750 dilution monoclonal antibody to mouse LOX-1 for overnight at 4°C. Membranes were washed and then incubated with 1:5000 dilution specific secondary antibody (Amersham Life Science) for 2 hours at room temperature, and the membranes were washed and detected with the ECL system (Amersham Life Science). The relative intensities of protein bands were analyzed by Scan-gel-it software (24).

Data Analysis

All data represent mean of duplicate samples. Data are presented as mean ± SD. Statistical significance was determined in multiple comparisons among independent groups of data in which ANOVA and the F test indicated the presence of significant differences. A P value <0.05 was considered significant.

Results

The synergistic anti-atherosclerotic effect of candesartan and rosuvastatin

Compared with the control mice (C57BL/6J mice fed regular diet), the apo-E knockout mice fed high-cholesterol diet developed extensive atherosclerosis ($P<0.01$ vs control mice). Although both candesartan and rosuvastatin alone decreased the extent of atherosclerosis ($p<0.05$ vs high-cholesterol diet alone), the combination reduced atherosclerosis to a much greater extent ($P<0.05$ vs candesartan or rosuvastatin alone plus high-cholesterol diet). Figure 1 shows results of representative experiments and the extent of atherosclerosis (mean ± SD) in different groups of animals.

Candersartan and rosuvastatin alone decreased atherosclerosis by about 35% and 25% respectively. The combination reduced atherosclerosis by 70%, demonstrating a synergistic effect.

The synergistic effect of candesartan and rosuvastatin on LOX-1 expression

In the control C57BL/6J mice, the expression of LOX-1 (mRNA and protein) was low (Figure 2). In contrast, LOX-1 expression (mRNA and protein) was markedly increased by high-cholesterol diet in apo-E knockout mice ($P<0.01$ vs control mice). Both candesartan and rosuvastatin alone decreased the LOX-1 expression (mRNA and protein), albeit modestly ($P<0.05$ vs high-cholesterol diet alone). The combination of candesartan and rosuvastatin had a dramatic inhibitory effect on the up-regulation of LOX-1 (mRNA and protein) in apo-E knockout mice ($P<0.01$ vs high-cholesterol diet alone).

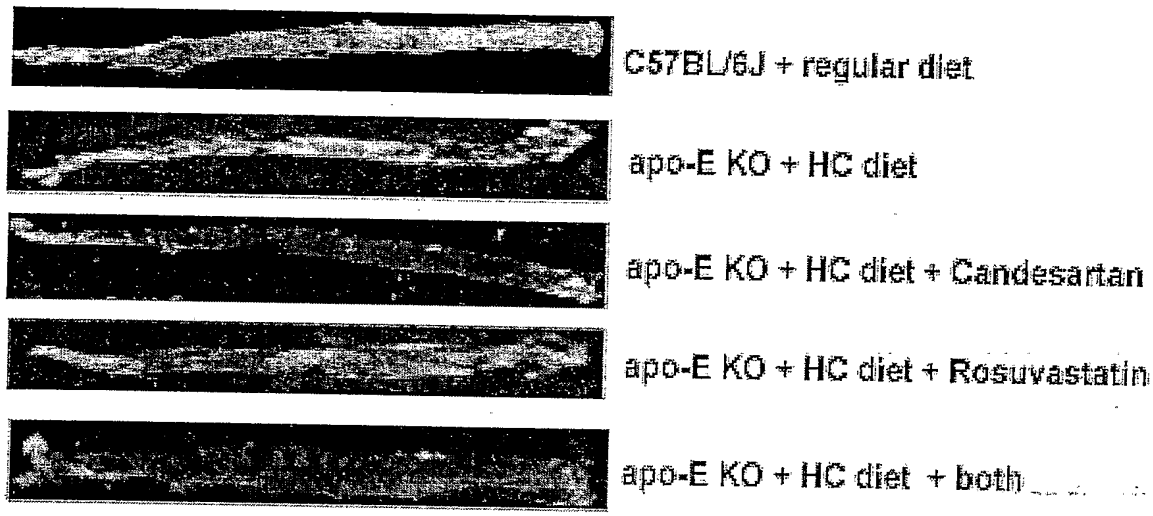
The synergistic effect of candesartan and rosuvastatin on CD40 expression

Compared with the expression in control C57BL/6J mice, CD40 expression (mRNA and protein) was markedly increased in apo-E knockout mice fed a high-cholesterol diet in ($P<0.01$ vs control mice). Although candesartan and rosuvastatin treatment alone slightly decreased CD40 expression ($P<0.05$ vs high-cholesterol diet alone), the combination of candesartan and rosuvastatin had a dramatic inhibitory effect on the up-regulation of CD40 (mRNA and protein) in the apo-E knockout mice ($P<0.01$ vs high-cholesterol diet alone).

The synergistic effect of candesartan and rosuvastatin on MMPs expression

Compared with the expression in control C57BL/6J mice, MMP-1, -2 and -9 expression (mRNA and protein) was markedly increased in high-cholesterol diet-fed apo-E knockout mice ($P<0.01$ vs control mice). Both candesartan and rosuvastatin alone decreased MMP-1, -2 and -9 expression (mRNA and protein), albeit modestly ($P<0.05$ vs high-cholesterol diet alone). The combination of candesartan and rosuvastatin had a dramatic inhibitory effect on their expression ($P<0.01$ vs high-cholesterol diet alone).

Figure 1


C57BL/6J + regular diet
apo-E KO + HC diet
apo-E KO + HC diet + Candesartan
apo-E KO + HC diet + Rosuvastatin
apo-E KO + HC diet + both

Claims

1. A combination comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof for prevention or treatment of atherosclerosis.

2. A pharmaceutical composition which comprises a combination as claimed in Claim 1 in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

3. A pharmaceutical composition which comprises a combination as claimed in Claim 1, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention of cardiovascular events.

4. A combination as claimed in Claim 1 for use in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

5. A combination as claimed in Claim 1 for use in the manufacture of a medicament for the prevention of cardiovascular events.

6. A combination as claimed in Claim 1 for use as a medicament for the prevention or treatment of atherosclerosis.

7. A combination as claimed in Claim 1 for use as a medicament for prevention of cardiovascular events.

8. A method of preventing or treating atherosclerosis in a warm-blooded animal, such as man, which comprises administering a combination as claimed in Claim 1.

9. Amethod of preventing cardiovascular events in a warm-blooded animal, such as man, which comprises administering a combination as claimed in Claim 1.

10. A kit comprising a combination as claimed in Claim 1; optionally with instructions for use in the prevention or treatment of atherosclerosis.

11. A combination as claimed in Claim 1 wherein candesartan is in the form of candesartan cilexetil.

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(71) Applicant *(for all designated States except US)*: **ASTRAZENECA UK LIMITED** [GB/GB]; 15 Stanhope Gate, London, Greater London W1K 1LN (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **MEHTA, Jay, Lal** [US/US]; University of Arkansas for Medical Sciences, 4301 W Markham, Slot 532, Little Rock, AR 72205 (US).

(74) Agent: **ASTRAZENECA**; Global Intellectual Property, S-151 85 Sodertalje (SE).

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(54) Title: THERAPEUTIC TREATMENT

(57) Abstract: A combination comprising candesartan and rosuvastatin for the prevention or treatment of arteriosclerosis and for the prevention of cardiovascular events is described.

THERAPEUTIC TREATMENT

The present invention relates to a combination comprising candesartan and rosuvastatin.

The present invention further relates to pharmaceutical compositions comprising the combination mentioned hereinbefore. The present invention further relates to the use of a combination mentioned hereinbefore in the prevention or treatment of atherosclerosis.

Atherosclerosis is a condition mediated by complex pathological processes which result in irregularly distributed lipid deposits in the arteries and is a major contributory factor to coronary heart disease. A reduction in atherosclerosis is therefore a major target for reducing the number of cardiovascular events for example, myocardial infarction, worsening of angina, cardiac arrest, stroke, congestive heart failure and cardiovascular death.

Dyslipidemia, particularly increased plasma level of low-density lipoprotein (LDL) is one of the major risk factors in atherosclerosis. Clinical studies have demostrated that reducing plasma LDL level with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, commonly known as statins, results in a lower risk of cardiovascular events.

Activation of the renin-angiotensin system (RAS) may be considered another important risk factor in atherosclerosis. Activation of RAS with the formation of angiotensin (II) (A (II)) and the activation of A (II) receptors have been implicated in atherogenesis, plaque rupture, myocardial ischemic dysfunction and congestive heart failure (Singh and Mehta, Arch Intern Med, 2003, vol 163, 1296-1304).

International Patent Application WO 95/26188 discloses treatment of atherosclerosis with A(II) receptor blockers, optionally in combination with HMGCoA reductase inhibitors. International Patent Application WO 01/76573 discloses the use of a combination of at least two of an A(II) antagonist, an ACE (angiotensin converting enzyme) inhibitor and an HMGCoA reductase inhibitor for the prevention or delay of progression in a list of conditions, amongst which is atherosclerosis.

We have surprisingly found that the combination of the A(II) antagonist candesartan and the HMG CoA reductase inhibitor rosuvastatin has a synergistic effect in the reduction of atherosclerosis. This synergistic effect appears to arise from synergistic inhibition of expresssion of a number of inflammatory mediators involved in the RAS (for example CD40, metalloproteinases (MMPs)) and/or inhibition of the expression of the receptor LOX-1 (which

is a receptor for oxidised LDL on endothelial cells). The synergistic effect provides strong evidence for cross-talk between the RAS and dyslipidemia in atherogenesis.

It will be appreciated that the activty of MMPs may be regulated in-vivo by their tissue inhibitors (TIMPs). We have also shown that the expression of TIMP-1 and TIMP-2 is up-regulated by high-cholesterol diet, and markedly attenuated by the combination of candesartan and rosuvastatin. These data lend credence to the concept that the balance between MMPs and TIMPs is altered by high-cholesterol diet, and that this imbalance can be "normalized" by the combination of an A(II) antagonist and a lipid-lowering agent.

In one aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for the prevention or treatment of atherosclerosis.

In one aspect of the invention is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for the prevention of cardiovascular events.

Such a combination may also be useful in the treatment or prevention of other diseases associated with these mediators, for example in inflammatory diseases or conditions, such as ischemia-reperfusion injury (to the heart, brain, kidneys, lungs and liver), radiation-induced injury, burn injury and peripheral vascular disease,

Candesartan may suitably be in the form of candesartan, or in the pro-drug form candesartan cilexetil. These forms may be formulated with a further agent such as a diuretic such as hydrochlorothiazide (for example, as marketed as Atacand Plus™).

Where herein candesartan is referred to, this includes both candesartan and candesartan cilexetil.

Preferably the calcium salt of rosuvastatin, which may be referred to as rosuvastatin calcium, is used in the various aspects of the present invention.

In general, pharmaceutically-acceptable salts include acid addition salts such as methanesulfonate, tosylate, α-glycerophosphate, fumarate, hydrochloride, citrate, maleate, tartrate and (less preferably) hydrobromide. Pharmaceutically-acceptable salts in general also include salts formed with phosphoric and sulfuric acid. Pharmaceutically-acceptable salts generally include base salts such as an alkali metal salt for example sodium, an alkaline earth metal salt for example calcium or magnesium, an organic amine salt for example triethylamine, morpholine, N-methylpiperidine, N-ethylpiperidine, procaine, dibenzylamine, N,N-dibenzylethylamine, tris-(2-hydroxyethyl)amine, tris(hydroxymethyl)methylammonium,

N-methyl d-glucamine and amino acids such as lysine. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions.

Herein, where the term "combination" is used it is to be understood that this refers to simultaneous, separate or sequential administration. In one aspect of the invention "combination" refers to simultaneous administration. In another aspect of the invention "combination" refers to separate administration. In a further aspect of the invention "combination" refers to sequential administration. Where the administration is sequential or separate, the delay in administering the second component should be such that both agents are present in the body so as to produce the synergistic effect of the combination.

In a further aspect of the invention is provided a pharmaceutical composition which comprises candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

In a further aspect of the invention is provided a pharmaceutical composition which comprises candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or reduction of risk of cardiovascular events.

The compositions described herein may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream, for rectal administration for example as a suppository or the route of administration may be by direct injection into the tumour or by regional delivery or by local delivery. In other embodiments of the present invention the compounds of the combination treatment may be delivered endoscopically, intratracheally, intralesionally, percutaneously, intravenously, subcutaneously, intraperitoneally or intratumourally. In general the compositions described herein may be prepared in a conventional manner using conventional excipients or carriers that are well known in the art.

Suitable pharmaceutically-acceptable excipients or carriers for a tablet formulation include, for example, inert excipients such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or alginic acid; binding agents such as gelatin or starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl 4-hydroxybenzoate, and

anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid excipient, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Candesartan is commercially available as 'Atacand™' and 'Atacand Plus™'. Rosuvastatin calcium is commercially available as 'Crestor™'. Suitable formulations for the present invention include those which are commercially available.

Suitable dosages of each component of the combination are those of the marketed commercial products. Alternatively, the synergy between the components may allow a lower dosage of one or both components to be used. For example, a dose of 4mg, 8mg, 16mg, 32mg, or up to 160mg of candesartan in combination with a dose of 80mg, 40mg, 20mg, 10mg, 5mg or 2.5mg of rosuvastatin may be used. It will be understood that any one of the doses of candesartan may be combined with any suitable dose of rosuvastatin.

In one aspect, 80mg of rosuvastatin is used. In another aspect, 40mg of rosuvastatin is used. In a further aspect, 20mg of rosuvastatin is used. In a further aspect, 10mg of rosuvastatin is used. In a further aspect, 5mg of rosuvastatin is used. In a further aspect, 2.5mg of rosuvastatin is used.

In one aspect, between 32 and 160mg, such as about 64 to 128mg, for example 64 to 112mg, such as about 64-96mg of candesartan is used. Conveninelty, about 72mg of candesartan is used. In another aspect, 32mg of candesartan is used. In a further aspect, 16mg of candesartan is used. In a further aspect, 8mg of candesartan is used. In a further aspect, 4mg of candesartan is used.

It will be appreciated that the pharmaceutical composition according to the present invention includes a composition comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier. Such a composition, for example in a single oral formulation conveniently provides the therapeutic combination product of the invention for simultaneous administration in the prevention or treatment of atherosclerosis.

Preferably the two components of the combination are both administered orally.

Preferably the two components of the combination are administered as a single oral formulation.

Preferably the combination is formulated for once-a-day dosing.

Conveniently, the combination is formulated as a single tablet or capsule.

The dosages and schedules described hereinbefore may be varied according to the particular disease state and the overall condition of the patient. For example, it may be necessary or desirable to reduce the above-mentioned doses of the components of the combination treatment in order to reduce toxicity. Dosages and schedules may also vary if, in addition to a combination treatment of the present invention, one or more additional chemotherapeutic agents are used. Scheduling can be determined by the practitioner who is treating any particular patient using his professional skill and knowledge.

A pharmaceutical composition according to the present invention also includes separate compositions comprising a first composition comprising candesartan or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier, and a second composition comprising rosuvastatin or a pharmaceutically acceptable salt thereof and a pharmaceutically-acceptable excipient or carrier. Such a composition conveniently provides the therapeutic combination of the invention for sequential or separate administration in the synergistic prevention or treatment of atherosclerosis but the separate compositions may also be administered simultaneously.

In another aspect of the invention there is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use as a medicament for the prevention or treatment of atherosclerosis.

In another aspect of the invention there is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use as a medicament for the prevention of cardiovascular events.

In another aspect of the invention there is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament.

In another aspect of the invention there is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a

pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

In another aspect of the invention there is provided a combination comprising candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for the prevention of cardiovascular events.

In a further aspect of the invention there is provided a method of preventing or treating atherosclerosis in a warm-blooded animal, such as man, which comprises administering a combination of candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof.

In a further aspect of the invention there is provided a method of preventing cardiovascular events in a warm-blooded animal, such as man, which comprises administering a combination of candesartan, or a pharmaceutically acceptable salt thereof, and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According to a further aspect of the present invention there is provided a kit comprising a combination of candesartan or a pharmaceutically acceptable salt thereof, and rosuvastatin; or a pharmaceutically acceptable salt thereof ,optionally with instructions for use in the prevention or treatment of atherosclerosis.

According to a further aspect of the present invention there is provided a kit comprising:

a) candesartan in a first unit dosage form;

b) rosuvastatin in a second unit dosage form; and

c) container means for containing said first and second dosage forms; and optionally

d) with instructions for use in the prevention or treatment of atherosclerosis.

According to another aspect of the present invention there is provided a method of inhibiting expression of CD40 and/or metalloproteinases (MMPs) by administering a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof.

Particular metalloproteinases are MMP-1, MMP-2 and MMP-9.

According to another aspect of the present invention there is provided a method of treating atherosclerotic patients by inhibition of expression of CD40 and/or metalloproteinases (MMPs) by administering an amount of a combination of candesartan, or a

pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof suitable for inhibition of expression of CD40 and/or metalloproteinases (MMPs).

According to a further aspect of the invention, there is provided a method for normalizing the balance between MMPs and TIMPS by administration of an amount of a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According to another aspect of the present invention there is provided a method of inhibiting expression of LOX-1 by administering a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof.

According to another aspect of the present invention there is provided a method of treating atherosclerotic patients by inhibition of expression of LOX-1 by administering an amount of a combination of candesartan, or a pharmaceutically acceptable salt thereof and rosuvastatin, or a pharmaceutically acceptable salt thereof suitable for inhibition of expression of LOX-1.

Materials and Methods

Animal Model

Five pairs of C57BL/6J mice and three pairs of homozygous apo-E knockout mice (on C57BL/6J background) were obtained from Jackson Laboratories (Bar Harbor, ME). They were bred by brother-sister mating and housed in a room lit from 6:00 AM to 6:00 PM and kept at 21°C. The C57BL/6J mice (n=10) were continued on regular diet for the entire study period. The apo-E knockout mice were divided into four groups. Group 1 (n=10) animals were given high-cholesterol diet (1% cholesterol) alone for 12 weeks since the age of 6 weeks; Group 2 (n=10) animals were given high-cholesterol diet with candesartan (1mg/kg/d) for 12 weeks since the age of 6 weeks; Group 3 (n=10) animals were given high-cholesterol diet with the rosuvastatin (1mg/kg/d) for 12 weeks since the age of 6 weeks; Group 4 (n=10) animals were given high-cholesterol diet with candesartan (1mg/kg/d) and rosuvastatin (1mg/kg/d) for 12 weeks since the age of 6 weeks.

At the end of 12-week-treatment, the mice were sacrificed and subject to studies described below. All experimental procedures were performed in accordance with protocols approved

by the Institutional Animal Care and Usage Committee of University of Arkansas for Medical Sciences.

Quantitative Analysis of Atherosclerotic Plaques

At the end of 12-week-treatment, 5 mice from each group were euthanized and the aortas were separated from surrounding tissues. After removal of the adventitial fat tissue, the aortas were opened longitudinally from the aorta arch to the iliac bifurcation, and fixed in 10% formalin for 24 hours. Then the aortas were rinsed in 70% alcohol briefly, stained with Sudan IV solution for 15 minutes, differentiated in 80% alcohol for 20 minutes and washed in running water for 1 hour (Russell L. Techniques for studying atherosclerotic lesion, Lab Invest. 1958; 7:42-47). The aortas were mounted and their pictures were taken with a camera connected to a dissection microscope. The images were analyzed by soft ware (Image Pro Plus, Media Cybernetics).

RNA Preparation and Analysis by RT-PCR

At the end of 12-week-treatment, 5 mice from each group were euthanized and the aortas (from aorta arch to iliac bifurcation) were separated from surrounding tissues and stored on dry ice. Each aorta was cut into four segments, two of which were used to extract total RNA with the single-step acid-guanidinium thiocyanate-phenol-chloroform method as described earlier (27). One microgram of total RNA was reverse transcripted into cDNA with oligo-dT (Promega, Madison, WI, U.S.A.) and Maloney murine leukemia virus (M-MLV) reverse transcription (Promega) at 42°C for 1 hour. Two microliters of reverse transcription (RT) material was amplified with Taq DNA polymerase (Promega) and a primer pair specific to mouse LOX-1, CD40 or MMPs (MMP-1, -2, -9). For mouse LOX-1, forward primer: 5′-TTACTCTCCATGGTGGTGCC-3′, reverse primer: 5′-AGCTTCTTCTGCTTGTTGCC-3′ were used. 30 cycles of polymerase chain reaction (PCR) were performed at 94°C for 40 seconds (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). The size of polymerase chain reaction (PCR) product was 193 base pairs. For mouse CD40, forward primer 5′-GTTTAAAGTCCCGGATGCGA-3′ and reverse primer 5′-CTCAAGGCTATGCTGTCTGT-3′ were used. 35 cycles of polymerase chain reaction (PCR) were performed at 94°C for 1 minute (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). The size of PCR product was 408 base pairs. For mouse MMP-1, forward primer 5′-GGACTCTCCCATTCTTAATGA T-3′ and reverse primer 5′-

CCTCTTTCTGGATAACATCATCA AC-3′ were used. For mouse MMP-2, forward primer 5′-ATCAAGGGGATCCAGGAGC-3′ and reverse primer 5′-GCAGCGATGAAG ATGATAG-3′ were used. For mouse MMP-9, forward primer 5′-AGTTTGGTGTCGCGGAGCAC-3′ and reverse primer 5′-TACATGAGCGCTTCCGGCAC-3′ were used. For all MMPs, 35 cycles of PCR were performed at 94°C for 1 minute (denaturation), 58°C for 1 minute (annealing), and 75°C for 1 minute (extension). The sizes of PCR product were 627, 718 and 753 base pairs, respectively. A primer pair specific to mouse β-actin was used as housekeeping gene (forward primer: 5′-TTCTACAATGAGCTGCGTTG-3′, reverse primer: 5′-CACTGTGTTGGCATAGAGGTC-3′). 30 cycles were used at 94°C for 30 seconds (denaturation), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension). PCR product was 560 base pairs. The reverse transcription PCR (RT-PCR)-amplified sample was visualized on 1.5% agarose gel using ethidium bromide.

Protein Preparation and Analysis by Western Blot

Each mouse aorta was cut into four segments. Two of them were used to extract RNA, and the remaining two were used to extract protein as described previously (14). In brief, the aortic tissues were homogenized and lysed in lysis buffer, then centrifuged at 4000 rpm for 10 minutes at 4°C. The lysate proteins from aortas (20 μg/lane) were separated by 10% SDS-PAGE, and transferred to nitrocellulose membranes. After incubation in blocking solution (5% non-fat milk, Sigma), membranes were incubated with 1:750 dilution monoclonal antibody to mouse LOX-1, 1:500 dilution polyclonal antibody to mouse CD40 (Santa Cruz), 1μg/ml dilution monoclonal antibody to mouse MMP-1 (Oncogene), 1μg/ml dilution monoclonal antibody to mouse MMP-2 (Oncogene), 1μg/ml dilution monoclonal antibody to mouse MMP-9 (Oncogene), 1:500 dilution polyclonal antibody to mouse TIMP-1 (Santa Cruz), 1:500 dilution polyclonal antibody to mouse TIMP-2 (Santa Cruz), or 1:5000 dilution monoclonal antibody to mouse β-actin (Sigma) for overnight at 4°C. Membranes were washed and then incubated with 1:5000 dilution specific secondary antibody (Amersham Life Science) for 2 hours at room temperature, and the membranes were washed and detected with the ECL system (Amersham Life Science). The relative intensities of protein bands were analyzed by Scan-gel-it software (Li DY, Zhang YC, Sawamura T, Mehta JL. Circ Res. 1999; 84:1043-1049).

Data Analysis

All data represent mean of duplicate samples. Data are presented as mean ± SD. Statistical significance was determined in multiple comparisons among independent groups of data in which ANOVA and the F test indicated the presence of significant differences. A P value <0.05 was considered significant.

Results

The synergistic anti-atherosclerotic effect of candesartan and rosuvastatin

Compared with the control mice (C57BL/6J mice fed regular diet), the apo-E knockout mice fed high-cholesterol diet developed extensive atherosclerosis (P<0.01 vs control mice). Although both candesartan and rosuvastatin alone decreased the extent of atherosclerosis (p<0.05 vs high-cholesterol diet alone), the combination reduced atherosclerosis to a much greater extent (P<0.05 vs candesartan or rosuvastatin alone plus high-cholesterol diet). Figure 1 shows results of representative experiments and the extent of atherosclerosis (mean ± SD) in different groups of animals.

Candersartan and rosuvastatin alone decreased atherosclerosis by about 35% and 25% respectively. The combination reduced atherosclerosis by 70%, demonstrating a synergistic effect. This effect is illustrated graphically in Figure 2.

The synergistic effect of candesartan and rosuvastatin on LOX-1 expression

In the control C57BL/6J mice, the expression of LOX-1 (mRNA and protein) was low. In contrast, LOX-1 expression (mRNA and protein) was markedly increased by high-cholesterol diet in apo-E knockout mice (P<0.01 vs control mice). Both candesartan and rosuvastatin alone decreased the LOX-1 expression (mRNA and protein), albeit modestly (P<0.05 vs high-cholesterol diet alone). The combination of candesartan and rosuvastatin had a dramatic inhibitory effect on the up-regulation of LOX-1 (mRNA and protein) in apo-E knockout mice (P<0.01 vs high-cholesterol diet alone).

The synergistic effect of candesartan and rosuvastatin on CD40 expression

Compared with the expression in control C57BL/6J mice, CD40 expression (mRNA and protein) was markedly increased in apo-E knockout mice fed a high-cholesterol diet in (P<0.01 vs control mice). Although candesartan and rosuvastatin treatment alone slightly

decreased CD40 expression (P<0.05 vs high-cholesterol diet alone), the combination of candesartan and rosuvastatin had a dramatic inhibitory effect on the up-regulation of CD40 (mRNA and protein) in the apo-E knockout mice (P<0.01 vs high-cholesterol diet alone).

The synergistic effect of candesartan and rosuvastatin on MMPs expression

Compared with the expression in control C57BL/6J mice, MMP-1, -2 and -9 expression (mRNA and protein) was markedly increased in high-cholesterol diet-fed apo-E knockout mice (P<0.01 vs control mice). Both candesartan and rosuvastatin alone decreased MMP-1, -2 and -9 expression (mRNA and protein), albeit modestly (P<0.05 vs high-cholesterol diet alone). The combination of candesartan and rosuvastatin had a dramatic inhibitory effect on their expression (P<0.01 vs high-cholesterol diet alone).

The effect of candesartan and rosuvastatin on TIMPs expression

TIMP-1 and TIMP-2 protein expression was also increased in apo-E knockout mice by high-cholesterol diet (P<0.01 vs. control mice), but the increase was less than that of MMPs. Both candesartan and rosuvastatin alone reduced TIMP-1 and TIMP-2 expression by a small degree (P<0.05 vs. high-cholesterol diet alone), but the combination of candesartan and rosuvastatin had a greater inhibitory effect on their expression (P<0.01 vs. high-cholesterol diet alone, P<0.05 vs. high-cholesterol diet with candesartan or rosuvastatin).

Claims

1. A combination comprising candesartan or a pharmaceutically acceptable salt thereof and rosuvastatin or a pharmaceutically acceptable salt thereof for prevention or treatment of atherosclerosis.

2. A pharmaceutical composition which comprises a combination as claimed in Claim 1 in association with a pharmaceutically acceptable diluent or carrier for use in the prevention or treatment of atherosclerosis.

3. A pharmaceutical composition which comprises a combination as claimed in Claim 1, in association with a pharmaceutically acceptable diluent or carrier for use in the prevention of cardiovascular events.

4. A combination as claimed in Claim 1 for use in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

5. A combination as claimed in Claim 1 for use in the manufacture of a medicament for the prevention of cardiovascular events.

6. A combination as claimed in Claim 1 for use as a medicament for the prevention or treatment of atherosclerosis.

7. A combination as claimed in Claim 1 for use as a medicament for prevention of cardiovascular events.

8. A method of preventing or treating atherosclerosis in a warm-blooded animal, such as man, which comprises administering a combination as claimed in Claim 1.

9. A method of preventing cardiovascular events in a warm-blooded animal, such as man, which comprises administering a combination as claimed in Claim 1.

10. A kit comprising a combination as claimed in Claim 1; optionally with instructions for use in the prevention or treatment of atherosclerosis.

11. A combination as claimed in Claim 1 wherein candesartan is in the form of candesartan cilexetil.

Figure 1

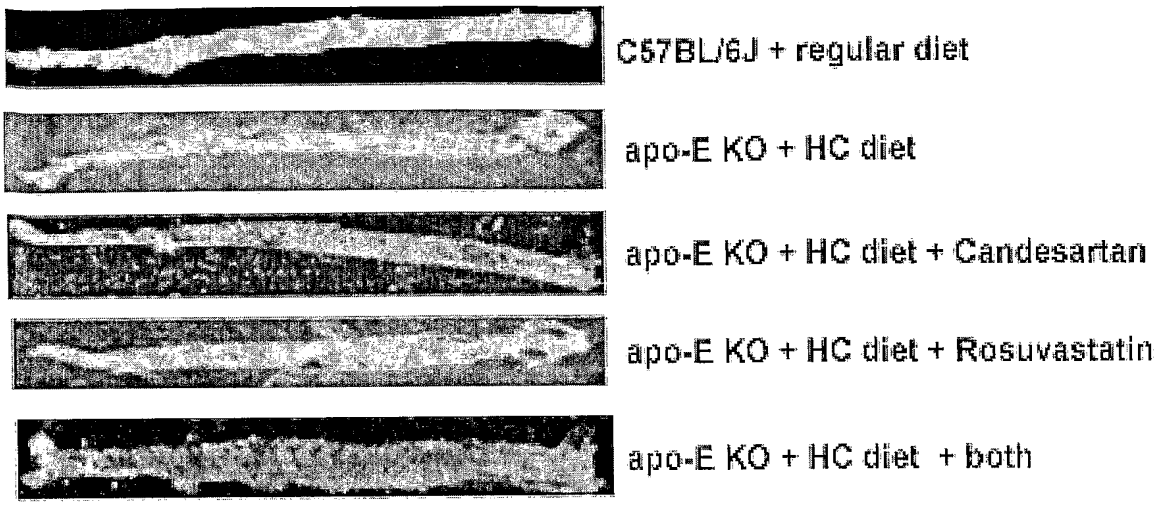
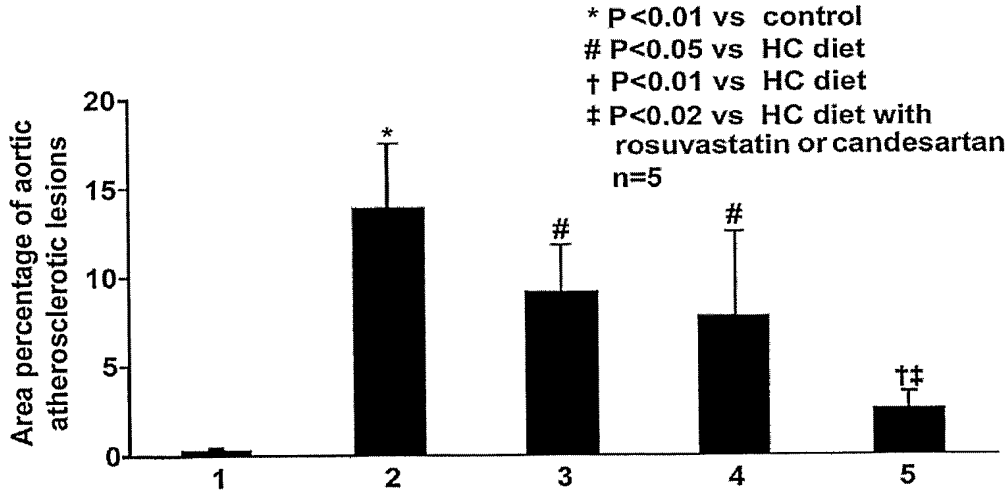


Figure 2



1. Control mice fed with regular diet
2. Apo-E KO mice fed with HC diet
3. Apo-E KO mice fed with HC diet together with rosuvastatin
4. Apo-E KO mice fed with HC diet together with candesartan
5. Apo-E KO mice fed with HC diet together with rosuvastatin and candesartan